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Human stem cell-derived astrocytes and their application to studying Nrf2-mediated neuroprotective pathways and therapeutics in neurodegeneration

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Glia, including astrocytes, are increasingly at the forefront of neurodegenerative research for their role in the modulation of neuronal function and survival. Improved understanding of underlying disease mechanisms, including the role of the cellular environment in neurodegeneration, is central to therapeutic development for these currently untreatable diseases. In these endeavours, experimental models that more closely reproduce the human condition have the potential to facilitate the transition between experimental studies in model organisms and patient trials. In this review we discuss the growing role of astrocytes in neurodegenerative diseases, and how astrocytes generated from human pluripotent stem cells represent a useful tool for analyzing astrocytic signalling and influence on neuronal function.

Introduction

Neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease, amyotrophic lateral sclerosis (ALS) and Huntington's disease are untreatable conditions that collectively represent a major healthcare burden. Improved understanding of the biology of these diseases is required in order to develop neuroprotective and ultimately reparative treatments. While these disorders differ in their symptomatology and presentation, they share some common features: gradual clinical progression over years, implicating ongoing degenerative processes, and disturbance of the cellular environment play a key role in neuronal deterioration [1]. Moreover, these disorders share some injury mechanisms. From human pathological tissue and rodent models of neurodegeneration, it is apparent

that oxidative stress, glutamate excitotoxicity and protein misfolding are involved in the progression of several disorders, both in the propagation of injury as well as potentially induction. In the context of oxidative stress, hallmarks of oxidative neuronal injury have been found in a range of disorders, including AD [2–4], ALS [5] and Parkinson's disease [6, 7]. Despite these established links between neurodegenerative disease to oxidative and nitrosative stress, trials of small molecule antioxidants or spin traps have had limited success [8]. There are many potential explanations for this, including the challenge of maintaining high concentrations of the drug in the brain in order to neutralize reactive oxygen species when they appear. Another consideration is that the antioxidant and detoxification systems of the brain are sophisticated and complex, and cannot be mimicked simply by a single small molecule. As

such, researchers are turning towards the mechanisms by which the brain's intrinsic antioxidant defences are controlled, or how neurons regulate downstream effects of oxidative insults, and how these may be manipulated for therapeutic effect [9–11].

The control of a neuron's antioxidant defences and indeed multiple aspects of its function are highly dependent on its cellular microenvironment, and in particular interactions between microglia and macroglia, including astrocytes [12]. Furthermore, neuronal dysfunction in several neurodegenerative disorders may be attributable in part to damage or dysfunction in astrocytes and other glial cells [13]. Human stem cell based technology now allows direct study *in vitro* of glial-neuronal interaction to study mechanisms of neurotoxicity and neuroprotection [14].

The growing role of astrocytes in neurodegenerative diseases

While traditional thinking has been neuron-centric in addressing the causes of neurodegenerative diseases, there is growing evidence for the role of glia, specifically astrocytes. It is becoming increasingly evident that the astrocytic environment can be central to disease outcome and, dependent on context, can be injurious or protective [15–19].

For example, in AD, reactive astrocytes are intimately associated with amyloid beta plaques in patient pathological tissue. Experimental studies have demonstrated that astrocytes undergo chemotaxis, responding to MCP-1 found in AD lesions, and internalize amyloid beta [20–22]. Astrocyte internalization of amyloid beta is potentially an ApoE-dependent process, which further implicates astrocytes in the pathogenesis of heritable forms of AD characterized by ApoE mutations [23]. The involvement of astrocytes in disease lesions has been replicated *in vivo*. Monitoring the migration of transplanted e-GFP positive astrocytes in human amyloid beta-bearing transgenic mice demonstrated that transplanted astrocytes migrated to and internalized amyloid beta [24]. Whilst contributing to clearance, amyloid beta internalization appears to detrimentally alter astrocyte behaviour causing accumulation of intracellular calcium, depletion of glutathione and mitochondrial dysfunction [25, 26]. Astrocytes appear to tolerate amyloid beta-induced metabolic changes reasonably well. However, co-cultured neurons die as a result. Mechanisms implicated in neurotoxicity include NADPH oxidase and PI3Kinase dysregulation [26, 27].

Alpha synuclein accumulation within astrocytes has been observed in the brains of Parkinson's disease patients and *in vitro* [28], where direct transfer of alpha-synuclein from neurons to astrocytes has been observed [29]. Expression of mutant alpha-synuclein selectively in astrocytes has been shown to result in neuronal death due to astro-

cytic dysfunction, evidenced by reactive gliosis, disruption of the blood–brain barrier, down-regulation of astrocyte glutamate transporters [30] and production of inflammatory cytokines [29].

Studies of the SOD1 transgenic model of ALS have provided strong evidence that astrocytes have a role in associated neuronal injury. Expression of mutant SOD1 specifically in mouse neurons on a wild-type background was insufficient to cause motor deficits in mice [31] and reduction of expression of mutant SOD1 specifically in astrocytes on a mutant background reduced disease progression [32]. In human mutant SOD1/wild-type SOD1 chimera mice, neurons bearing the mutation underwent differential survival according to neighbouring non-neuronal cells. If neighbouring astrocytes were wild-type, the neurons survived, and if the neighbouring astrocytes bore mutant SOD1, significant neuronal loss was noted. Moreover wild-type neurons with neighbouring mutant astrocytes also bore hallmarks of degeneration [15]. Rodent *in vitro* disease modelling using a 'mix and match' co-culture of wild-type and mutant astrocytes with motor neurons has extended these insights to suggest contact and soluble mediated mechanisms of mutant astrocyte-mediated neurotoxicity [19,33]. Similar insights were made in the human *in vitro* system. Human embryonic stem cell derived motor neurons were combined with rodent astrocytes [34] and human foetal astrocytes expressing SOD1 mutations [18], substantiating the 'neurotoxic' nature of mutant astrocytes. These studies further identified candidate molecules as mediators of injury, such as prostaglandin D₂, in murine astrocytes and allowed testing of candidate therapeutic agents [34]. The recent discovery of the role of TDP43 in sporadic [35, 36] and familial ALS [37], and FUS in familial ALS [38, 39] will serve to accelerate investigation into mechanisms of ALS pathogenesis and the potential further roles of astrocytes. Both TDP43 and FUS are nucleic acid binding proteins that play a role in regulation of gene transcription and splicing, presenting the possibility of common downstream pathological mechanisms underlying these forms of ALS. The role of astrocytes in brain function and disease is dealt with more fully in a number of recent reviews [13, 40, 41]

Increasing the translatability of model studies: the issue of species specific differences

Experimental and descriptive studies in model organisms such as rodents, non-mammalian vertebrates and invertebrates have contributed greatly to our understanding of the central nervous system, both in health and disease. Conservation of gene orthologues, functional anatomy and development of the central nervous system facilitates the translation of experimental findings between model organisms and humans. As a result, insights from these

studies have informed our understanding of disease mechanisms and revealed new therapeutic targets.

Nevertheless, important species specific differences have been identified at the cellular level, which have the potential to limit inferences made from rodent data to humans. Oberheim *et al.* reported that human astrocytes differed greatly from the rodent counterpart *in vivo*, with greater size, complexity of arborization, number of subtypes, GFAP expression and speed of calcium wave propagation, attesting to structural and functional differences [42]. Molecular level evidence suggests that gene regulation may differ more than expected between rodent and humans. A significant proportion of human transcription factor (TF) binding sites do not function in rodents [43]. Indeed, the conservation of TF occupancy in orthologous mouse and human gene promoters can be highly variable [44]. Of note, while interspecies differences in TF binding were large, the location of binding sites within different cellular human systems has been reported to be highly conserved [44]. Consistent with this, gene promoter sequence, as opposed to any differences in transcriptional machinery, appears to be the dominant factor directing species specific transcription [45], and strengthen the case for employing human-based systems to study transcriptional responses to enable the identification of interspecies differences and thus home in on the most human-relevant pathways. Beyond transcriptional differences, species specific differences in other aspects of molecular biology are also well-documented, including pathways relevant to neurophysiology and pathophysiology. For example, the developmental regulation of microtubule associated protein tau (*MAPT*, implicated in a group of disorders called tauopathies, which includes AD) isoforms differs between mice and humans, favouring four repeat tau in mouse and a combination of three and four repeat tau in humans [46], as well as differences in splicing of the $\text{Na}^+/\text{Ca}^{2+}$ transporter 1 (*NCX1*) [47], a class of pumps important for neuronal Ca^{2+} homeostasis under ischaemic conditions [48]. At the protein level, interaction partners, subcellular distribution and enzyme-substrate profiles can also differ. For example, the C-terminal PDZ ligand of human somatostatin receptor 3 binds the multi-PDZ-domain containing protein MUPP1 (a tight-junction protein proposed to regulate intracellular cell signalling pathways in a variety of tissues), unlike the rat receptor [49]. There exist human vs. rodent species specific differences in the way apoptotic caspases process their targets, and also in whether a protein is a substrate for caspases at all [50]. Of relevance to therapeutics is the fact that certain pharmacological compounds show species specific differences in efficacy such as those targeting the TRPV1 channel [51], the P2X2 receptor [52] and the TRPA1 receptor [53].

Several conclusions can be drawn from these studies, the most apparent being that significant differences can exist at the molecular and cellular level in humans and

rodents, and that rodent-based studies may benefit from complementary approaches using human cells of the appropriate type.

Human pluripotent stem cells as an experimental model

Rodent-human disparity increases the burden of proof that a particular finding has inter-species relevance, necessitating that certain findings be replicated in human experimental models [54–56]. Human *in vitro* platforms offer the opportunity to investigate disease processes and potential protective mechanisms in human cells. Three sources of human neural cells are readily identifiable, embryonic stem cell-derived, foetal-derived and those derived from the adult brain. For ethical and practical reasons, including limited propagation potential, unpredictable availability and inability to direct differentiation reliably, the use of human adult brain-derived material is limited as an expandable *in vitro* system. Human foetal-derived neural precursor cells (NPCs) can be derived from defined areas of the central nervous system (CNS) and expanded *in vitro* in the presence of neuroepithelial mitogens as non-adherent neurospheres [57, 58] or as adherent cultures [59]. Despite being well characterized, human foetal-derived NPCs are not ideal for long term study on account of practical reliability of tissue procurement, potentially limited differentiation ability to specific neuronal and glial subtypes and uncontrollable variability in sample gestation age [57].

In contrast, human embryonic stem cells (HESCs) are particularly attractive for experimental study, due to their predictable responsiveness to developmental cues enabling controlled and scaleable directed differentiation to neuronal and glial cell types. Following the isolation of HESCs [60], many methodologies now exist for successful maintenance of HESCs, generation of neural precursor cells and subsequent differentiation to defined neuronal subtypes [61–64], also comprising adherent and non-adherent neurosphere based systems. Adherent systems and selective propagation appear to allow greater cell yield, culture consistency and purity [65–67]. These systems in turn permit *in vitro* modelling of human neuronal injury and, along with recent reports of astrocyte generation from HESCs, further allow study of glial-neuronal interaction [54, 65]. Indeed HESC-derived motor neurons and dopaminergic neurons have been used to model glial toxicity and both antioxidant and GDNF-mediated neuroprotection [14, 18, 34].

Furthermore, insights from HESCs based experiments also inform and benchmark human induced pluripotent stem cell based studies. The development of induced pluripotent cells (iPSCs) permits the generation of defined neural cells from readily accessible patient material such as fibroblasts for study of hereditary disorders on the

Table 1

A summary of the strengths and weaknesses of rodent primary culture models compared to human embryonic cell line-based systems

Cell culture based <i>in vitro</i> system	Strengths	Weaknesses
Rodent culture	Comparative ease of derivation of mature cell types Genetic manipulation Animal models allow transplantation studies	Differences in biology may limit translation for human disease modelling
Human embryonic stem cell (HESC)	Human genome adds relevance Expandable in culture Can potentially derive many/all neural cell types in a single line	Technically difficult Currently enriched derivation of all mature neural cell types is limited Heterogeneity between different HESC cell lines Functional validation necessary

pre-existing human genetic background and as a potential source of patient-specific cells for autologous cell-replacement therapies. An example of *in vitro* adult brain disease modelling using patient specific material is that of Parkinson's disease-causing LRRK2 mutation carrying neurons having increased vulnerability to oxidative injury as well as morphological defects [68, 69]. This and other proof of concept studies illustrate the potential of human iPSC lines to accelerate disease understanding and drug development in a variety of neurological disorders [70–77].

The utilization of human embryonic stem cell lines with a known genetic background is of tremendous benefit with experimental reproducibility. However, it insufficiently reflects the genetic diversity inherent in the living human population. Similarly, genetic homogeneity within mouse strains, while inherently useful for knock-out studies and wild-type comparisons, insufficiently reflects natural population heterogeneity and observations magnified in a homogeneous genetic background may be diminished upon translation to heterogeneous populations, an observation conceptually distinct from human-non human model disparity as discussed earlier. Using a range of human *in vitro* lines may begin to address human genetic heterogeneity, and generate a platform observation rather than cell line specific observation. However, the range of cell lines required to account for human population variation completely is currently unknown, and may need to be developed on a sub-population basis. Overall, human stem cell-based approaches have distinct advantages and disadvantages compared with rodent systems (Table 1) and studies involving both systems may offer the best way of limiting confounds associated with any one experimental system.

Generating functional astrocytes from human stem cells

As outlined earlier, astrocytes perform key physiological processes vital to combating oxidative, excitotoxic and other forms of injury, and evidence suggests that dysfunction and impairment of these physiological processes

can significantly contribute to disease progression and outcome. Accordingly, modulating the cellular environment and specifically astrocyte function may play a role in slowing or even reversing neurological injury. Pre-requisites for detailed studies of neuronal–glial interaction in the context of human experimental models of neurological disease are characterized populations of enriched and functional human pluripotent stem cell (HPSC) derived neurons and astrocytes. Previous studies have focussed predominantly on the differentiation of HPSCs into non-specific and regionalized neurons for isolated study of neuronal injury. A range of systems has been described, from suspension to adherent culture methods [61, 78], and recent advances have made it possible to derive enriched neuron-biased neural precursor cells from HESCs, which can be readily specified to specific neuronal sub-types [79, 80]. In contrast, derivation of enriched functional human astrocytes from HESCs has historically received lesser attention, due to comparatively poor understanding of astrocyte specification during development and poor specificity of astrocyte precursor markers [81].

Progressive developments in technologies that allow maintenance and propagation of high quality neural precursors derived from HPSCs [61, 78, 80, 82, 83] and noting the temporal regulation of gliogenesis have established an experimental platform for scaleable generation of human astrocytes. Astrocyte differentiation is determined temporally by both intrinsic temporally mediated mechanisms and exogenous factors, including BMP4 and LIF [84]. LIF and other members of the IL-6 cytokine family have been shown to drive synergistically astroglial differentiation in conjunction with BMPs, STAT3 and SMAD1 converging upon CBP/p300 respectively [85]. Notch intracellular signalling also appears to have a contributory role [86]. However, while BMP and LIF signalling can drive astrocyte differentiation in glial-competent NPCs, their limited capacity to do so in early NPCs suggests that other intrinsic factors govern glial competence. The glial competence of NPCs increases over time, with early precursors being predominantly neuronal in fate and later precursors generating glia. This gliogenic switch appears to be regulated by

epigenetic changes resulting in chromatin remodelling and DNA methylation around the GFAP promoter and other astrocyte specific genes, to promote astrogliogenesis [87, 88]. The relevance of SMAD and STAT signalling to astroglial differentiation of HESC-derived neural precursor cells *in vitro*, however, remains unproven. The use of BMP4 in astroglial differentiation from human foetal-derived neural precursor cells provides some evidence that complementary pathways may exist [89]. Therefore, we systematically tested the ability of combinations of BMPs and LIF to drive astroglial conversion. Upon withdrawal of mitogens, drawing the neural precursor cells out of the cell cycle, application of combined Smad and Stat signalling mediators drove efficient differentiation of neural precursor cells to the astroglial lineage [65]. Other groups have similarly used prolonged culture of neural precursor cells and CNTF-mediated Stat signalling for astroglial conversion from HESCs [90]. Critically, glial-appropriate functionality of these *in vitro* human astrocytes was further demonstrated. Studies in rodent systems have demonstrated the role of astrocytes in glutamate clearance, expression of key astrocyte-specific proteins and the ability to modulate the synaptic maturation of co-cultured neurons. HESC-derived astrocytes accordingly demonstrated the acquisition of functional properties including glutamate uptake and expression of astrocyte markers GFAP, EAAT1, AQP4 and S100 β [65], calcium wave propagation and induction of neuronal synapses [90]. Critically, demonstration of these properties, shared with their *in vivo* counterparts, confirms astrocyte differentiation techniques and permits the investigation of interactions between human astrocytes and neurons. Future studies will be required to address whether HESC astrocytes also secrete the range of proteins expressed by murine astrocytes, including clusterin and thrombospondin [91–93]. Recent advances have demonstrated a panoply of astrocytic functions in rodent systems, including functional gliotransmission with consequent modulation of homosynaptic and heterosynaptic neurotransmission in hippocampal networks by astrocytes [94], and direct control of sleep pressure [95] and regulation of breathing in response to acidaemia by astrocytes [96]. It remains to be seen whether human embryonic stem cell derived astrocytes recapitulate these and other *in vivo* properties and are able to integrate functionally into these networks upon transplantation, a key property ahead of potential transplantation therapies.

The neuroprotective abilities of astrocytic Nrf2 in rodent and human models

Given the importance of oxidative injury in neurological diseases, understanding the mechanisms underlying neuronal oxidative cell death and endogenous antioxidant

mechanisms may provide tractable therapeutic targets. The nuclear factor erythroid-2-related factor 2 (Nrf2, encoded by *NFE2L2*) pathway has been identified for its key role in mediating the cellular antioxidant protective response and thus as a potential candidate for targeted therapeutics in neurodegenerative disease. Nrf2 is a member of the cap'n'collar basic leucine zipper family of transcription family, and is widely regarded as the master regulator of antioxidant defences and drug metabolizing enzymes [97]. Under normal conditions Nrf2 is bound in the cytoplasm by Keap1 and targeted for degradation. However inhibition of Keap1-dependent degradation allows Nrf2 to translocate to the nucleus and activate transcription of antioxidant response element (the cis-acting promoter element, ARE) containing genes [98]. Various mechanisms appear to underlie Nrf2 activation, both direct and indirect. Nrf2 can be activated directly by oxidative stress caused by peroxide treatment and oxygen-glucose deprivation [54] and indirectly. Loss of p62 has been associated with development of AD-like pathology in rodent models; p62 knock-out mice accumulate markers of oxidative damage with age, and age-correlated accumulation of p62 promoter damage has been demonstrated in human and murine samples [99]. p62 has been shown to associate with Keap1 and cause disinhibition of Nrf2 [100]. As such heritable or acquired loss of p62 may cause accumulation of cellular oxidative injury due to dysregulation of Nrf2 activity.

Important ARE-containing genes include phase II detoxifying and antioxidant enzymes, which are largely dependent on Nrf2-mediated activation upon injury. Critical enzymes include haem oxygenase 1, sulfiredoxin, peroxiredoxins and those central to glutathione (GSH) synthesis and metabolism including GCL and GST [101, 102]. Activation of Nrf2 by genetic manipulation, hypoxia/ischemia or small chemical activators has been shown to abate neurological injury and disease progression in rodent systems [54, 103–109]. The Nrf2 pathway is particularly active in astrocytes, compared with neurons (although activation of Nrf2 in neurons is possible [110]), and activation of astrocytic Nrf2 is sufficient to confer neuroprotection on nearby neurons via a mechanism proposed to involve the release of glutathione [103, 105, 111] (Figure 1). Although driving Nrf2 expression in neurons is strongly neuroprotective [112], astrocytic expression of Nrf2 confers neuroprotection in models of Parkinson's disease, ALS and ischaemia [103, 104, 111, 113].

The use of small molecule activators of Nrf2, which act by antagonizing Keap1-mediated Nrf2 degradation, represents a promising form of antioxidant therapy. Previous therapeutic strategies have primarily focussed on compounds that directly combat ROS, such as natural antioxidants (e.g. vitamin E) and synthetic spin traps [8]. These have enjoyed considerable efficacy in animal model studies but have been translationally disappointing, potentially due to the difficulty in getting sufficient quantities of

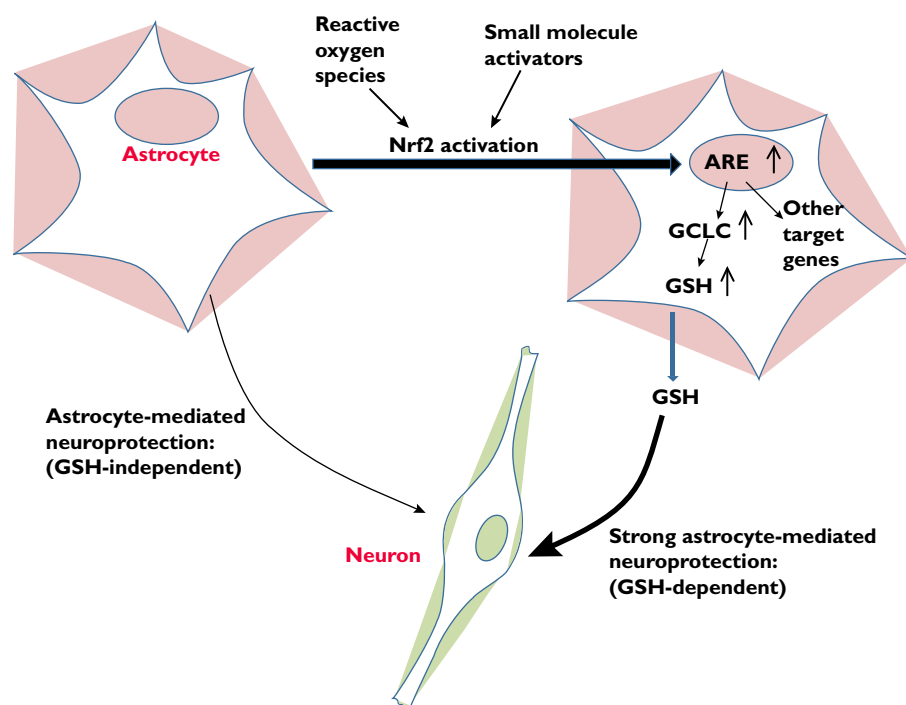


Figure 1

Humanembryonic stem cell-derived astrocytes mediate non-cell autonomous neuroprotection in part via activation of Nrf2-mediated gene expression. See text and reference [65] for details

antioxidants into the human brain to have an effect [8]. Rather than acting as free radical scavengers or spin traps themselves, these molecules act by boosting Nrf2-regulation, thus up-regulating the intrinsic antioxidant defences of cells. Among small molecule Nrf2 inducers, the series of synthetic oleanane triterpenoids, such as derivatives of 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO) developed by Michael Sporn (Dartmouth Medical School), have received much attention due to their potency as well as low toxicity in animal studies [114, 115]. Outside of the CNS, CDDO compounds are potently protective in a variety of disorders, including inflammatory lung disorders, cancer (prevention and treatment), renal and hepatic toxicity, and diabetes [116]. Equally impressive is their efficacy in models of a variety of neurodegenerative disorders. In mouse models of Huntington's disease chronic administration of CDDO-ethylamide and CDDO-trifluoroethylamide (CDDO^{TFEA}) upregulated Nrf2 target genes, attenuated striatal atrophy and improved the *in vivo* phenotype exhibited, including motor performance by rotorod testing and survival [107]. Three month oral administration of CDDO reduced plaque and microglial burden in a mutant APP transgenic mouse model of AD, with concomitant improvement in spatial memory by Morris water maze [117]. Moreover, CDDO compounds also have demonstrable protective effects in *in vivo* models of Huntington's disease and Parkinson's disease [118] as well as the G93A SOD1 model of ALS [116].

Given that the neuroprotective effects of CDDO-triterpenoids have been well established in rodent systems, we sought to use CDDO-triterpenoid mediated neuroprotection as a proof-of-concept to illustrate the utility of our HESC-derived platform to investigate astrocyte-mediated neuroprotection. We demonstrated that HESC-derived astrocytes respond physiologically to CDDO^{TFEA}-triterpenoid treatment, upregulating glutathione-mediated antioxidant processes, and protect HESC-derived neurons from peroxide-mediated cell death by soluble factors secreted into astrocyte-conditioned medium [65]. This protective effect was in addition to the basal protection mediated by conditioned medium from untreated astrocytes (Figure 1). Furthermore direct CDDO^{TFEA}-triterpenoid treatment conferred no protective benefit to HESC-derived neurons against oxidative cell death, demonstrating the non-cell autonomous mechanisms involved in CDDO^{TFEA}-triterpenoid mediated neuroprotection [65]. These findings suggest that HESC-derived neural derivatives can be used effectively to model human neuron-astrocyte interactions, investigate neurological injury and rescue mechanisms, and have the potential to increase the translational hit of findings made in complementary non-human based systems. Moreover, similarly derived HESC-derived astrocytes also respond to mild oxidative stress by inducing Nrf2 target genes [54], raising the possibility that Nrf2 forms an endogenous adaptive protective response in the CNS as well.

Concluding remarks

Developments in HESC-based technologies permit studies of early neuronal development, modelling neural injury *in vitro*, and potentially cell-replacement therapies. Amongst other injury mechanisms, oxidative stress is a common theme in the aetiopathogenesis of a range of chronic neurodegenerative disorders. As a result, informed antioxidant strategies that alter redox balance effectively may contribute to novel therapies across a range of conditions, and multi-modal therapies may be of benefit. Furthermore, astrocytes have been proven to be principle sites of oxidative-stress mediated neuronal injury and neuroprotection as well as the therapeutic target for candidate drugs. As such, non-cell autonomous neuroprotection, as shown in these studies, has widespread and fundamental implications for human-based drug discovery and screening of novel neuroprotective agents. Screens focussing solely on the direct effect of compounds on isolated neurons may overlook potentially important neuroprotective processes that act via astrocytes or other non-neuronal cells. Despite these advances, inferences drawn from *in vitro* models to the *in vivo* state are limited by the cell types included in the co-culture. Pure neuron-astrocyte co-cultures lack the complexity that neighbouring oligodendrocytes and microglia would present *in vivo*. Furthermore, the reduced culture time and as yet unclear correlation of *in vitro* culture time points with *in vivo* development suggest that *in vitro* models may encounter difficulty in recapitulating aspects of long term chronic neurodegeneration. Nevertheless, human *in vitro* systems provide unparalleled access to human cell types and have the potential to provide previously inaccessible insights in cell-cell interaction, molecular processes and potentially tractable clinical targets. Beyond disease modelling, the generation of enriched human neurons and astrocytes offers the prospect of cell replacement therapies. Many hurdles remain, including the enriched generation of subtype-specific cells, issues surrounding tissue rejection and functional engraftment in appropriate disease models. Some experimental successes have been achieved with engraftment of HESC-derived dopaminergic neurons in rodent models of Parkinson's disease [119, 120]. Glial replacement therapies also offer some promise as neuroprotective interventions. Astrocyte replacement has already received some success in experimental rodent-based models of age-related neurodegeneration, spinal cord injury and ALS [17, 121]. The ability to alter the behaviour of these cells prior to implantation, either genetically [113, 122] or with drug treatment [65], may also enhance their neuroprotective abilities in the injured brain. Longer term strategies building on these insights that target mobilization of endogenous glial populations are an area of particularly active research.

In summary the establishment of bespoke human *in vitro* platforms for neurological disease modelling and

drug discovery offers a major new resource to accelerate successful clinical translation of novel neuroprotective therapeutics.

Competing Interests

There are no competing interests to declare.

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